SUPPORTING INFORMATION

Proteins in Action: Femtosecond to Millisecond Structural Dynamics of a Photoactive Flavoprotein

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Figure S1.

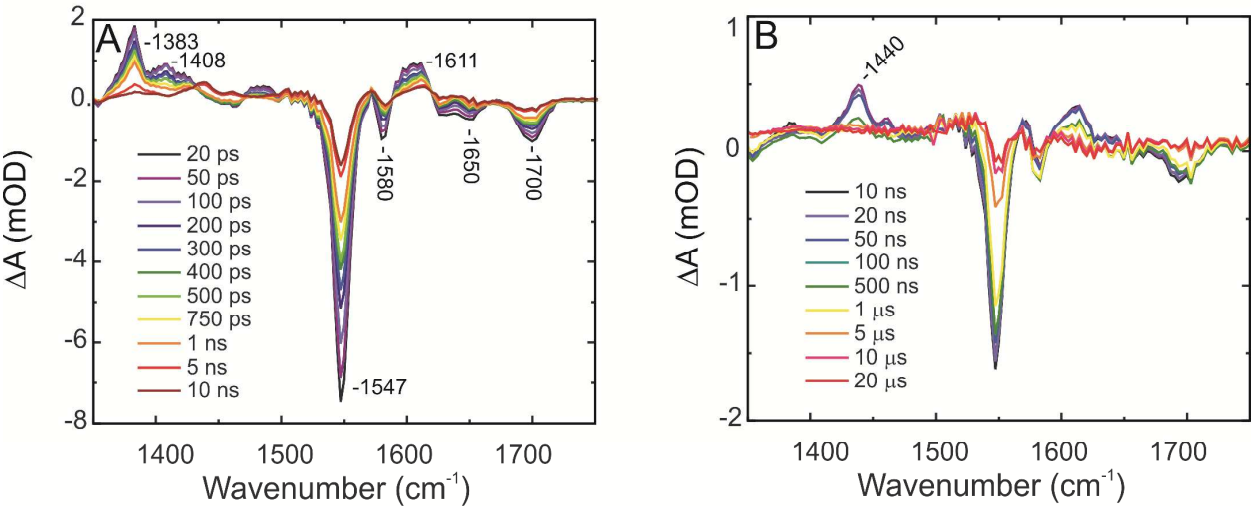

Figure S1. **Time resolved IR difference spectra for FMN** (*A*) recorded between 20 ps and 10 ns after excitation of the flavin in aqueous solution at 450 nm. (*B*) Relaxation in the FMN spectrum between 10 ns and 20 μs after excitation. FMN was chosen over FAD as in aqueous solutions there is a reaction between the flavin ring and the adenine which is absent in the protein. The band which appears near 1440 cm^{-1} in FMN is tentatively assigned to the triplet state, which has completely decayed in < 5 μs. The observation of this mode for FMN in aqueous solution suggests the need to consider triplet contributions in wtAppA_{BLUF} (main Figure 2).

Figure S2.

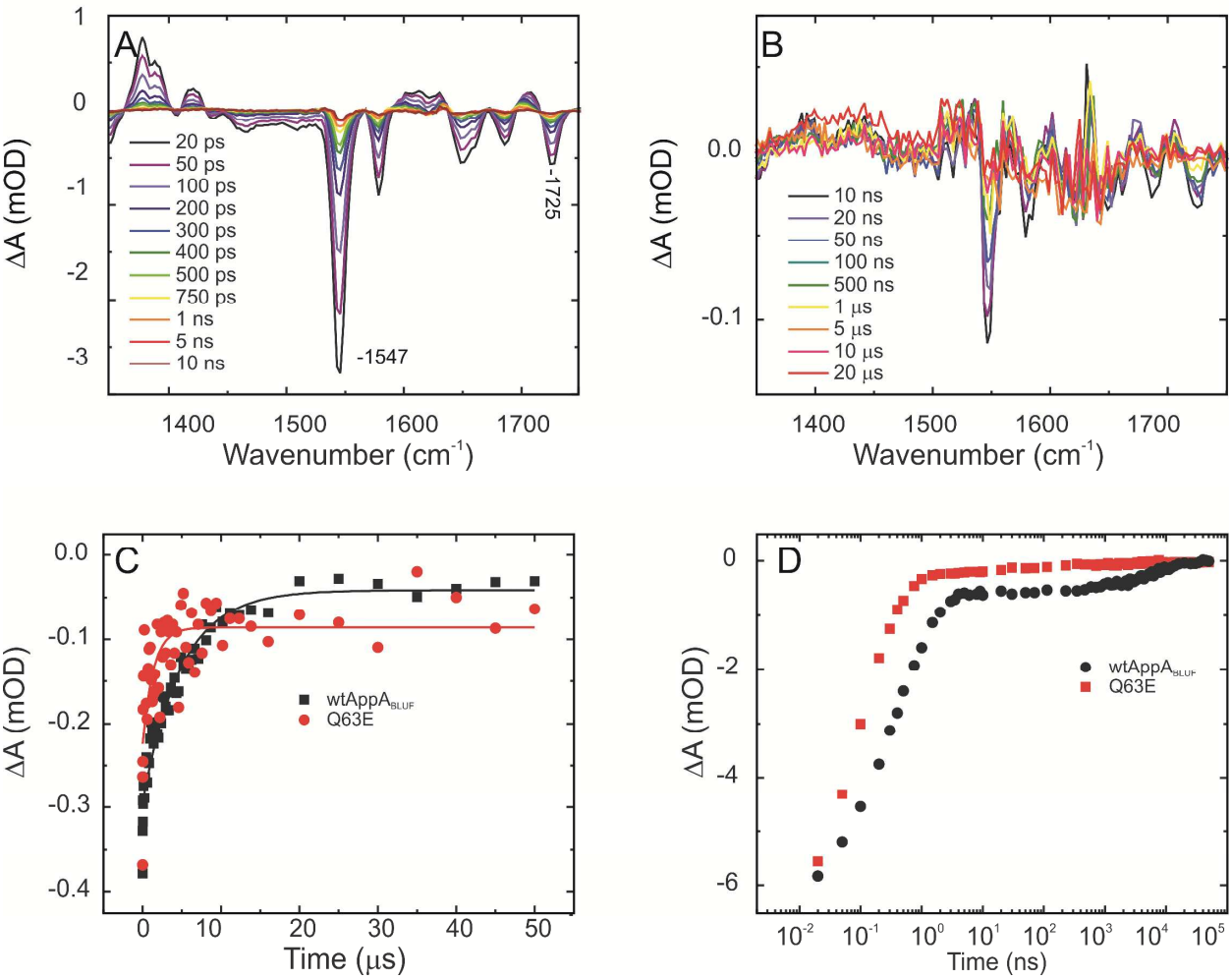

Figure S2. **Comparison of TRIR for Photoactive AppA and the photoinactive Q63E** (*A*) Time resolved IR difference spectra for Q63E $AppA_{BLUF}$ recorded between 20 ps and 10 ns after excitation of FAD at 450 nm. The dominant process is clearly relaxation to repopulate the ground state and no obvious triplet formation is observed. Note that in this photoinactive mutant the protein modes seen in AppA (main text Figure 2) do not develop. (*B*) Relaxation in the Q63E $AppA_{BLUF}$ spectrum between 10 ns and 20 µs after excitation taken at 1547 cm^{-1}. (*C*). Transient kinetics on the microsecond timescale for $wtAppA_{BLUF}$ and Q63E. Q63E is known to be

photoinactive but also to reveals excitation induced perturbation of the protein modes (e.g. the 1725 cm^{-1} bleach). (*A*) and (*B*) both show the lack of formation of 1622/1631 cm^{-1} in Q63E. Both (*B*) and (*C*) show the fast decay of the photoinactive protein (possible due to a small population of the triplet state) and the relatively slower recovery of the photoactive protein, which we assign (main text) to reorganization in the surrounding protein. (*D*) The data of Figure 2C are shown on a log-time axis but including the ultrafast response to highlight the differences on both long and short timescales.

Table S1. **TRIR Kinetics of AppABLUF, W104A, and M106A determined by TRIR**.

Sample	α_1	τ_1/ps	α_2	τ_2/ps	$<\tau>$/ps
dAppA	-0.51	34 ± 4	-0.49	473 ± 73	249
lAppA	-0.78	11 ± 1	-0.28	134 ± 24	45
dW104A	-0.42	18 ± 1	-0.58	393 ± 18	236
lW104A	-0.64	11 ± 1	-0.36	91 ± 8	40
dM106A	-0.39	68± 29	-0.61	446 ± 50	298
lM106A	-0.73	11 ± 1	-0.27	150 ± 20	49

Protein samples were measured by TRIR at a concentration of 2 mM in pD 8 phosphate buffer. Under these conditions in the 50 μm pathlength flow cell use for the measurements the IR transmission was typically 50%. The ground state recovery kinetics (1547 cm^{-1}) were fit to a sum of two exponentials; the α_i are weightings connected to the relaxation times, τ_i and $<\tau>$ is a weighted average relaxation time. Designation l and d are for light and dark adapted forms. The fast relaxation of the light adapted state will be discussed elsewhere. Note that the two exponential function certainly describes the kinetics on a fast timescale, but other functions may be possible, and it is likely that the observations are consistent with a distribution of decay times reflecting an inhomogeneous distribution of ground state structures.

Figure S3

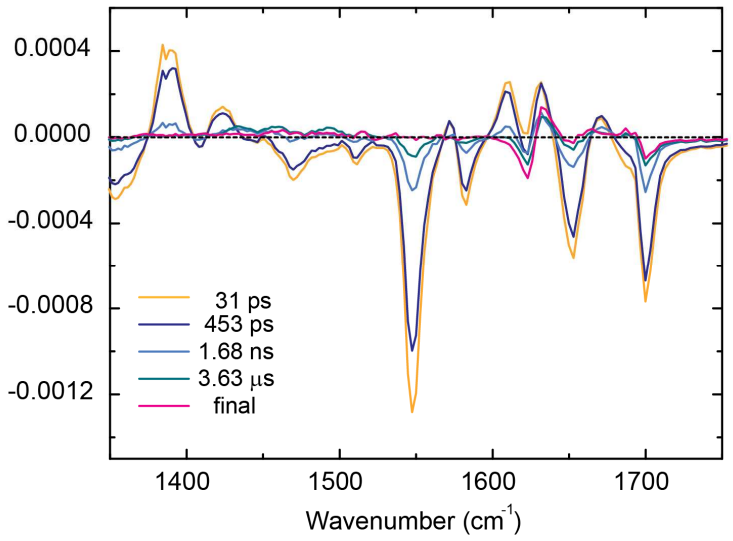

Figure S3. **Global Analysis of dAppA.** We subjected the data set of Figure 2A and B to a global kinetic analysis.[S1] In this analysis an initial, three intermediate and a final spectrum were assumed to be connected by sequential first order kinetics. As expected the chromophore kinetics dominate the early time data, relaxing in two subnanosecond steps to a nanosecond lived state. These reproduce the non-single exponential kinetics of the chromophore discussed in the main text. This state evolves in microseconds to a final state. The resulting 'evolution associated spectra' reproduce the temporal evolution of the protein modes at 1622/1631 cm^{-1}, with the single time constant representing an average of the individual kinetics reported in Table 1. Additional intermediates produce different decay times but do not improve the quality of the fit. The full exploitation of global analysis methods requires still better signal-to-noise.

Figure S4.

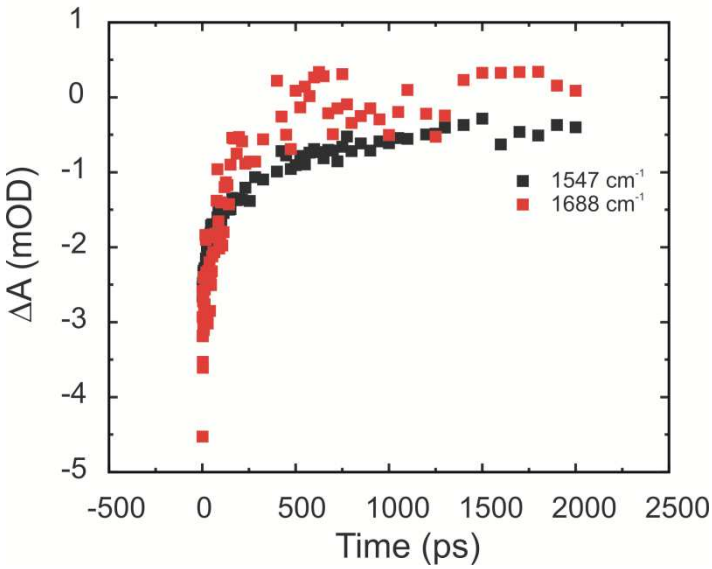

Figure S4 **Kinetics for W104A**. A The sub-nanosecond appearance of the C4=O carbonyl transient (1688 cm^{-1}, red) mirrors that of the ground state recovery e.g. measured from the isoalloxazine ring mode at 1547 cm^{-1} (black).

Figure S5.

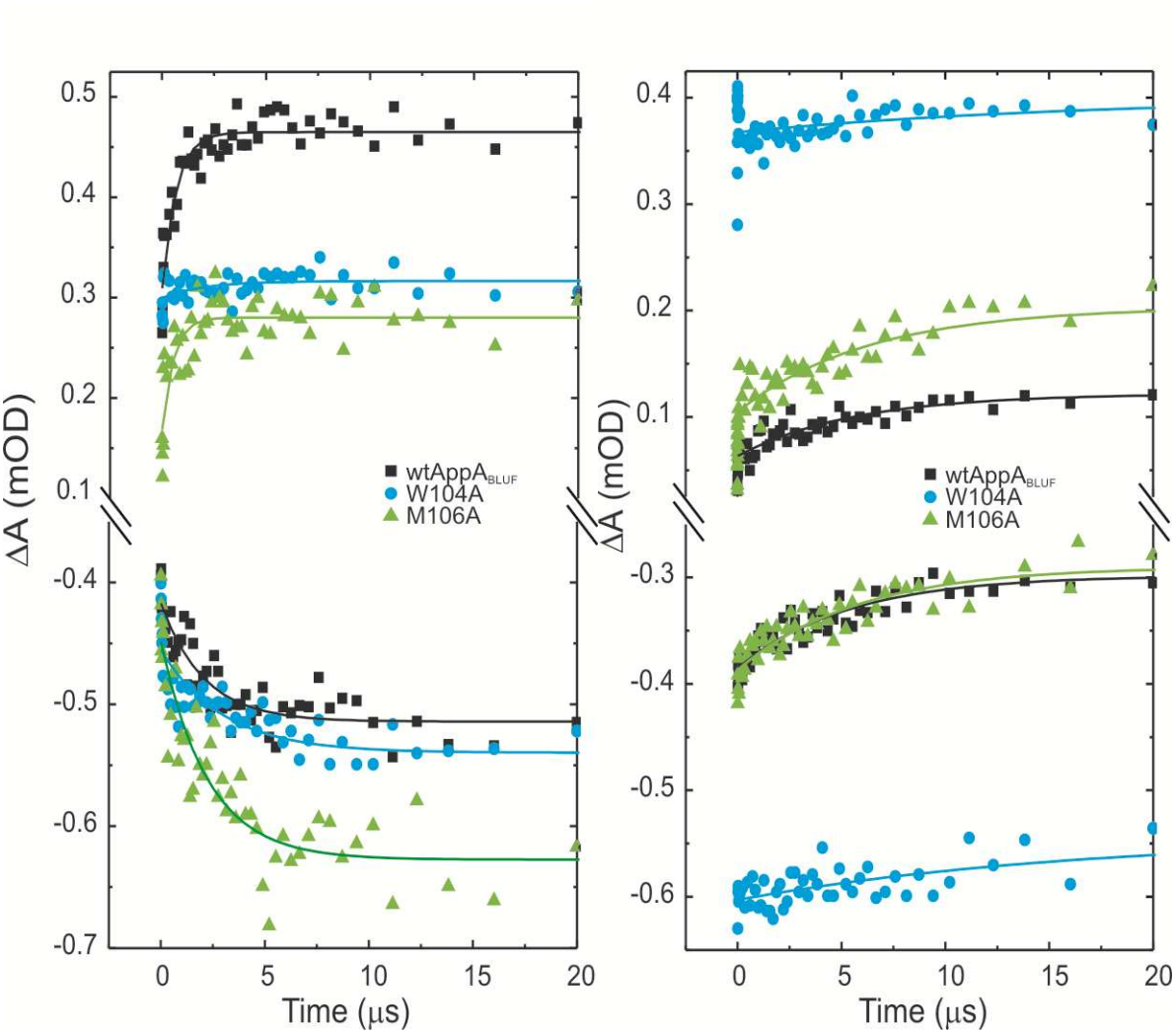

Figure S5. **Transient kinetics associated with protein and C4=O modes** in W104A compared with M106A and dAppA$_{BLUF}$ (*A*) Transient dynamics in the protein modes at 1622 (bottom) and 1631 cm^{-1} (top) showing the similarity of timescales for dAppA$_{BLUF}$ and M106A and the distinct kinetics for W104A. (*B*) Transient dynamics at mode associated with reorganization about the C4=O carbonyl chromophore modes 1688 (top) and 1703 cm^{-1} (bottom) for the dAppA$_{BLUF}$ and the two mutants.

References

S1. (a) van Stokkum, I. H. M.; Larsen, D. S.; van Grondelle, R., Global and target analysis of time-resolved spectra. *Biochimica Et Biophysica Acta-Bioenergetics* **2004,** *1657* (2-3), 82-104; (b) Snellenburg, J. J.; Laptenok, S. P.; Seger, R.; Mullen, K. M.; van Stokkum, I. H. M., Glotaran: A Java-Based Graphical User Interface for the R Package TIMP. *Journal of Statistical Software* **2012,** *49* (3), 1-22.